

CLAIMS:

1. An isolated polynucleotide encoding a polypeptide which includes the amino acid sequence shown in Figure 2.

5

2. A polynucleotide according to claim 1 wherein the coding sequence is the coding sequence shown in Figure 2.

3. A polynucleotide according to claim 1 wherein the coding sequence is a mutant, allele, variant or derivative of the coding sequence shown in Figure 2, by way of addition, deletion, substitution and/or insertion of one or more nucleotides.

4. An isolated polynucleotide which on expression in a transgenic plant exerts a negative regulatory effect on a pathogen defence response of the plant, which defence response is pathogen independent and autonomous of the presence of pathogen, the polynucleotide encoding a polypeptide which includes an amino acid sequence which is a mutant, allele, variant or derivative of the Barley *Mlo* sequence shown in Figure 2, or is a homologue of another species or a mutant, allele, variant or derivative thereof, the amino acid sequence differing from that shown in Figure 2 by way of addition, substitution, deletion and/or insertion of one or more amino acids.

10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95

109

5. A polynucleotide according to claim 4 encoding a polypeptide which includes the amino acid sequence shown in Figure 13.

5 6. A polynucleotide according to claim 5 wherein the coding sequence is that shown in Figure 10.

7. A polynucleotide according to claim 5 wherein the coding sequence is a mutant, allele, variant or derivative of the coding sequence shown in Figure 10, by way of addition, deletion, substitution and/or insertion of one or more nucleotides.

8. A polynucleotide according to claim 4 encoding a polypeptide which includes the amino acid sequence shown in Figure 14.

9. A polynucleotide according to claim 8 wherein the coding sequence is that shown in Figure 11.

20 10. A polynucleotide according to claim 8 wherein the coding sequence is a mutant, allele, variant or derivative of the coding sequence shown in Figure 11, by way of addition, deletion, substitution and/or insertion of one or more nucleotides.

25 11. A polynucleotide according to claim 4 encoding a

10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95

110

polypeptide which includes the amino acid sequence shown in Figure 15.

12. A polynucleotide according to claim 11 wherein the coding
5 sequence is that shown in Figure 12.

13. A polynucleotide according to claim 11 wherein the coding sequence is a mutant, allele, variant or derivative of the coding sequence shown in Figure 12, by way of addition, deletion, substitution and/or insertion of one or more nucleotides.

14. A polynucleotide according to any preceding claim operably linked to a regulatory sequence for expression.

15

卷之三

20

15. An isolated polynucleotide encoding a polypeptide which on expression in a transgenic plant produces a polypeptide which can stimulate or maintain a defence response of the plant, the encoded polypeptide including an amino acid sequence which is a mutant, allele, variant or derivative of the Barley Mlo sequence shown in Figure 2 or of a homologue of another species, the amino acid sequence differing from that shown in Figure 2 by way of addition, substitution, deletion and/or insertion of one or more amino acids.

16. A polynucleotide according to claim 15 which stimulates or maintains said defence response of the plant on homozygous

expression in the plant.

17. A polynucleotide according to claim 15 wherein the amino acid sequence includes an alteration identified in Table 1.

5

18. A polynucleotide according to claim 17 wherein the amino acid sequence is that of Figure 2 including a substitution at residue 240.

10

19. A polynucleotide according to claim 17 wherein the amino acid sequence includes Leucine at residue 240.

15

20. A polynucleotide according to any of claims 15 to 19 operably linked to a regulatory sequence for expression.

20

21. An isolated polynucleotide which has at least about 600 contiguous nucleotides of the nucleotide sequence of any of claims 1 to 13 or complement thereof

25

22. A polynucleotide according to claim 21 operably linked to a regulatory sequence for transcription.

25

23. An isolated polynucleotide which has at least about 300 contiguous nucleotides of the sequence of any of claims 1 to 13, or complement thereof, operably linked to a regulatory sequence for transcription.

24. A polynucleotide according to claim 22 or claim 23 wherein the regulatory sequence includes an inducible promoter.

25. A nucleic acid vector suitable for transformation of a host cell and including a polynucleotide according to any preceding claim.

26. A nucleic acid vector according to claim 25 wherein said host cell is a microbial cell.

27. A nucleic acid vector according to claim 25 wherein said host cell is a plant cell.

28. A host cell containing a heterologous polynucleotide or nucleic acid vector according to any preceding claim.

29. A cell according to claim 28 which is microbial.

30. A cell according to claim 28 which is a plant cell.

31. A cell according to claim 30 having said heterologous polynucleotide incorporated within its genome.

32. A cell according to claim 31 having more than one said polynucleotide per haploid genome.

33. A cell according to any of claims 30 to 32 which is

comprised in a plant.

34. A plant including a cell according to any of claims 30 to 32.

5

35. A plant which is a sexually or asexually propagated off-spring, clone or descendant of a plant according to claim 34, or any part or propagule of said plant, off-spring, clone or descendant.

10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25

36. A part or propagule of a plant according to claim 35.

37. A plant according to claim 34 which does not breed true.

38. A method of producing a plant, the method including incorporating a heterologous polynucleotide according to any of claims 1 to 14 into a plant cell and regenerating a plant from said plant cell.

20 39. A method of producing a plant, the method including incorporating a heterologous polynucleotide according to any of claims 15 to 20 into a plant cell and regenerating a plant from said plant cell.

25 40. A method of producing a plant, the method including incorporating a heterologous polynucleotide according to any of claims 21 to 24 into a plant cell and regenerating a plant from

said plant cell.

41. A method according to any of claims 38 to 40 including sexually or asexually propagating or growing off-spring or a 5 descendant of said plant.

42. A method of stimulating a defence response in a plant, the method including causing or allowing transcription from a heterologous polynucleotide according to any of claims 1 to 14 10 within cells of the plant.

43. A method of stimulating a defence response in a plant, the method including causing or allowing transcription from a heterologous polynucleotide according to any of claims 15 to 20 15 within cells of the plant.

44. A method of stimulating a defence response in a plant, the method including causing or allowing transcription from a heterologous polynucleotide according to any of claims 21 to 24 20 within cells of the plant.

45. A method of producing a polynucleotide encoding a polypeptide which on expression in a transgenic plant produces a polypeptide which can stimulate or maintain a defence 25 response of the plant, the method including alteration of the nucleotide sequence of a polynucleotide according to any of claims 1 to 14.

46. A method according to claim 45 involving site-specific sequence mutation.

47. A method according to claim 45 involving intracellular 5 homologous recombination.

48. A method wherein following alteration of a nucleotide sequence in accordance with the method of claim 45 a polynucleotide including the altered nucleotide sequence is introduced into a host cell.

49. A method according to claim 48 wherein the host cell is a plant cell.

50. A method wherein following introduction of a polynucleotide into a plant cell in accordance with claim 49 a plant is regenerated from the cell or descendants thereof including the altered nucleotide sequence.

51. Use of a polynucleotide according to any of claims 1 to 14 for stimulating a defence response in a plant.

52. Use of a polynucleotide according to any of claims 15 to 20 for stimulating a defence response in a plant.

25

53. Use of a polynucleotide according to any of claims 21 to 24 for stimulating a defence response in a plant.

54. Use of a polynucleotide according to any of claims 21 to 24 for down-regulation of expression of a gene encoded a polypeptide encoded by a polynucleotide according to any of claims 1 to 14.

5

55. Use of a polynucleotide according to any of claims 1 to 14 in the production of a transgenic plant.

10

56. Use of a polynucleotide according to any of claims 15 to 20 in the production of a transgenic plant.

15

57. Use of a polynucleotide according to any of claims 21 to 24 in the production of a transgenic plant.

20

58. A method of determining the presence of a pathogen resistance or susceptibility allele in a plant or plant cell, the method including analysing a sample from the plant or plant cell by:

(a) comparing the sequence of nucleic acid in the sample with all or part of the nucleotide sequence shown in Figure 7 to determine whether the sample from the patient contains a mutation;

(b) determining the presence in the sample of a polypeptide including the amino acid sequence shown in Figure 7 or a fragment thereof and, if present, determining whether the polypeptide is full length, and/or is mutated, and/or is expressed at the normal level;

5 (c) performing DNA fingerprinting to compare the restriction pattern produced when a restriction enzyme cuts nucleic acid in the sample with the restriction pattern obtained from the nucleotide sequence shown in Figure 7 or from a known mutant, allele or variant thereof;

10 (d) contacting the sample with a specific binding member capable of binding to nucleic acid including the nucleotide sequence as set out in Figure 7 or a fragment thereof, or a mutant, allele or variant thereof, the specific binding member including nucleic acid hybridisable with the sequence of Figure 7 or a polypeptide including a binding domain with specificity for nucleic acid including the sequence of Figure 7 or the polypeptide encoded by it, or a mutated form thereof, and determining binding of the specific binding member;

15 (e) performing PCR involving one or more primers based on the nucleotide sequence shown in Figure 7 to screen the sample for nucleic acid including the nucleotide sequence of Figure 7 or a mutant, allele or variant thereof.

20 59. A method of determining the presence of target nucleic acid in a plant or plant cell, the method including contacting a nucleic acid molecule which includes the nucleotide sequence shown in Figure 7 or an oligonucleotide fragment thereof with nucleic acid in a sample from the plant or plant cell and 25 assessing hybridisation of said nucleic acid molecule with nucleic acid in the sample.

60. A method according to claim 59 which involves amplification of nucleic acid to which said nucleic acid molecule hybridises.

5 61. A method according to claim 59 or claim 60 wherein said nucleic acid molecule includes an alteration in sequence compared with the nucleotide sequence shown in Figure 7 or corresponding fragment thereof.

10 62. A method according to claim 61 wherein said alteration is selected from those shown in Table 1.

15 63. An assay method for identifying a compound able to bind the polypeptide encoded by the polynucleotide of any of claims 1 to 14 or any of claims 15 to 20, the method including:

20 (a) bringing into contact said polypeptide or a fragment thereof, and a test compound; and
(b) determining interaction or binding between said polypeptide or fragment thereof and the test compound.

25

64. An assay method according to claim 63 wherein a compound is identified which is able to bind the polypeptide for which the amino acid sequence is shown in Figure 2.

65. An assay method for identifying a compound able to stimulate a defence response in a plant by interaction with the polypeptide encoded by the polynucleotide of any of claims 1 to

14 or any of claims 15 to 20, the method including:

(a) contacting a plant or plant part with a test compound and determining stimulation of a defence response; and

(b) bringing into contact said polypeptide or a fragment

5 thereof with a test compound and determining interaction or binding between said polypeptide or a fragment thereof and the test compound;

10 step (b) being performed with a test compound which tests positive in step (a), or step (a) being performed with a test compound which tests positive in step (b), or steps (a) and (b) being performed in parallel.

15 66. An assay method according to claim 65 wherein stimulation of a defence response is determined by monitoring pathogen growth and/or viability on the plant or plant part.

20 67. An assay method according to claim 65 or claim 66 wherein a compound is identified which is able to bind the polypeptide for which the amino acid sequence is shown in Figure 2.

68. An assay method according to any of claims 65 to 67 wherein a compound is identified which is able to stimulate resistance to powdery mildew in barley.

25 69. A method which includes following identification of a compound as being able to stimulate a defence response in a plant in accordance with any of claims 65 to 68 formulation of

the compound, or optionally if the compound is peptidyl nucleic acid encoding it, into a composition including at least one additional component.

5 70. A method which includes following identification of a compound as being able to stimulate a defence response in a plant in accordance with any of claims 56 to 58 application of the compound, or optionally if the compound is peptidyl nucleic acid encoding it, to a plant.

71. Use of a polypeptide encoded by a polynucleotide according to any of claims 1 to 14, in screening for compounds able to stimulate a defence response in a plant.

72. Use of a polypeptide encoded by a polynucleotide according to any of claims 15 to 20, in screening for compounds able to stimulate a defence response in a plant.

73. A compound able to stimulate a defence response in a plant
20 identified by a method according to any of claims 63 to 68.

add A \rightarrow